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TITLE: Branched Nerve Allografts to Improve Outcomes in Facial Composite Tissue Transplantation

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14. ABSTRACT

Background

The gold standard for peripheral nerve gap repair remains the autologous nerve graft. However, ''off-the-shelf'' alternatives are appealing due to additional graft material, shorter operative times and avoidance of donor site morbidity. This study aims to evaluate the functional and histologic recovery of a novel branched acellular nerve allograft (ANA) in a complex nerve defect.

Methods

Yucatan miniature swine (n = 13) underwent transection of the left inferior division of the facial nerve containing the marginal mandibular (MMB) and cervical (C) nerve branches. The gap was addressed either by a sural nerve autograft, ANA, or a human xenograft with or without oral Tacrolimus. Electrophysiologic assessments were performed pre-operatively and at the study endpoint (24 weeks) to assess functional recovery using compound muscle action potential (CMAP) and histologic recovery by immunohistochemistry (IHC) using neurofilament and S100.

Results

There were intrinsic functional differences between normal MMB (4.48±0.90 mV) and C (2.70±0.99 mV) nerves ($p<0.0005$). Nerve regeneration as seen with IHC and functional recovery occurred for all subjects. Functionally, there were no statistically significant differences between groups. Histologically, there was less degeneration and more organized fascicular anatomy in autograft and xenograft+Tacrolimus nerves (pending further analyses).

Conclusion

In this large animal model, novel branched ANA, human xenograft with and without Tacrolimus provide functional and histologic recovery in a complex facial nerve defect.

15. SUBJECT TERMS

Nerve regeneration, nerve repair, nerve gap, processed nerve allograft

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1. INTRODUCTION:

This project is a product development effort that will support the commercialization of a decellularized Branched Nerve Allograft to improve functional outcomes in Composite Tissue Transplantation (CTT) and the correction of branched nerve defects. The purpose is to facilitate CTT as a means to provide adequate restoration of sensorimotor function in patients with severe injuries to the faces, upper and lower limbs, abdominal wall and urogenital anatomy. Nerve repair and regeneration is currently a limiting aspect of CTT. Nerves re-grow at 1 cm/mo, and although immunosuppressants used in CTT can accelerate this rate, the return of function and sensation to the transplanted parts remains in the order of months to years. In addition, CTT interventions are a highly coordinated effort of multiple surgical teams working a tightly scheduled sequence of events to keep the donor tissues viable. Nerve dissection and repair, particularly of branched nerves adds significant time to the operation. Thus, branched nerves are a significant limiting factor to successful CTT of certain anatomical regions. CTT needs an off-the-shelf product that is immunologically tolerated, has versatile geometries and lengths, can be thawed and used as needed, and does not require additional surgical time for isolation of construction of the branched nerve graft. The Branched Decellularized Nerve Allograft will reduce the number of surgical sites and the duration of the surgical procedures while providing comparable or improved functional nerve regeneration with respect to the standard of care, thus resulting in improved outcomes.

2. KEYWORDS: nerve allografts; composite tissue transplantation; facial transplantation; nerve repair; branched nerves.

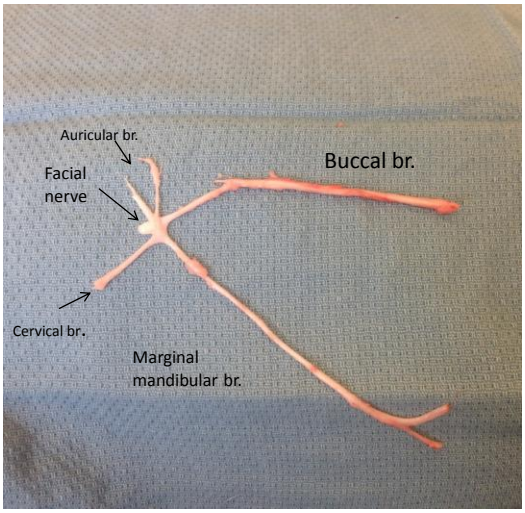
3. ACCOMPLISHMENTS:

What were the major goals of the project?

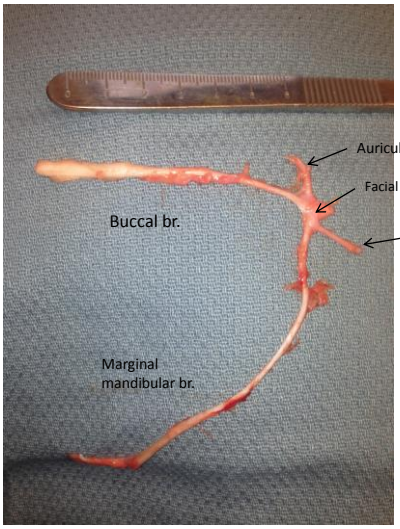
- 1) Swine Cadaver Facial Branched Nerve Dissection & Harvesting (Months 1-4)
- 2) Decellularization of Species Specific Branched Nerve Allograft (Months 4 – 14) and Product optimization, performed by subcontractor Axogen.
- 3) Simulated facial CTT in swine (i.e, surgery, electrophysiologic assessments, nerve explantation, histological assessments) (Months 12-24)
- 4) Analysis and Close out (Months 18-36 – ongoing)

- **What was accomplished under these goals?**
  - 1) major activities
    - As detailed in our prior reports, in our initial dissections, we discovered additional nerve branching in swine facial nerve when compared to the previous literature reports and were cited in our initial grant application (Sasaki R, Watanabe Y, Yamato M, Aoki S, Okano T, Ando T. Surgical anatomy of the swine face. Lab Anim 2010;44:359–363). We reported these findings in a peer-reviewed

journal (Aycart MA, Alhefzi M, Bueno E, Pomahac B. Surgical Anatomy of the Whole Facial Nerve for Enabling Craniofacial and Regenerative Medicine Translational Research in Swine. Journal of reconstructive microsurgery. 2015;31:547-50. PMID: 26115545). Select photos of nerve explantation are shown below.



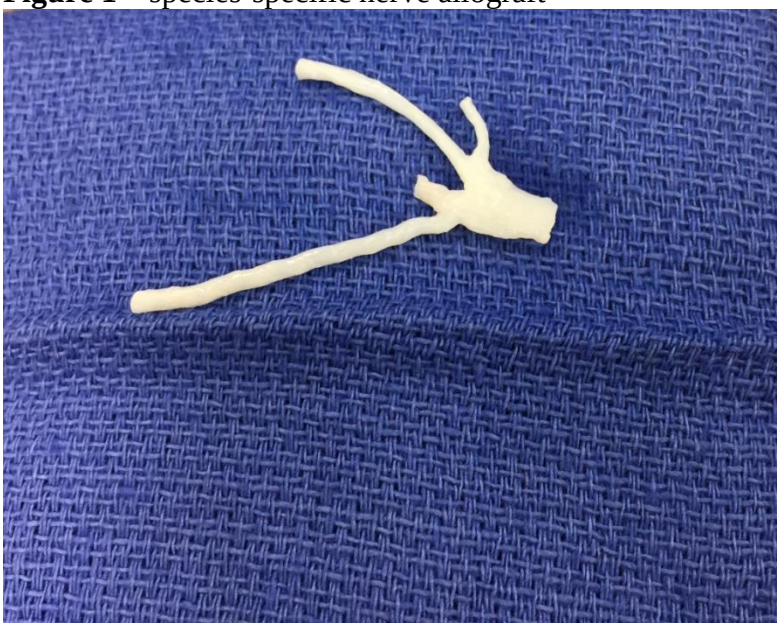
Right facial nerve



Left facial nerve

        - Based on these findings and our original proposal, we developed a branching facial nerve model in swine. We maintained the concept of a two-branch nerve allograft, only this time, we used the inferior division of the facial nerve, specifically, the cervical and marginal mandibular nerve branches.
    - Task 2 and 3. Decellularization of Species Specific Branched Nerve Allograft
        - Dr. Curt Deister and staff at AxoGen provided species –specific (see Figure 1 below) and human (xenografts) branched decellularized nerve grafts using the proprietary Avance® processing methods.

Figure 1 – species-specific nerve allograft



- Development of a sural nerve autograft nerve repair model:
    - **<u>Control (autograft nerve) animals used for model development:</u>**
    - Yucatan miniature swine (n = 5) underwent transection of the inferior division of the facial nerve. The inferior division of the facial nerve was sharply transected at a point 5mm proximal to the branching point of the marginal mandibular and cervical nerve branches. Approximately 25mm distal to the transection, the marginal mandibular and cervical nerves were isolated and sharply transected to create a 30mm nerve gap. Sural nerves were harvested from ipsilateral (same side of facial dissection) extremities. Gross electrophysiologic assessments were performed at study end point (24 weeks) to assess functional recovery by direct distal nerve stimulation prior to nerve harvest. Muscle-specific movement (e.g., lip and angle of the mouth depression for the marginal mandibular nerve and lateral neck flexion for the cervical) was considered a positive test at 2mA of electrical stimulation. Distal

nerve stumps were harvested for immunohistochemical evaluation using neurofilament (NF) and anti-S-100 to observe the distribution and presence of Schwann cells in the respective nerve fibers.

- After 24 weeks, sural nerve cable grafting provided functional marginal mandibular and cervical nerves across a 30mm branching nerve gap. Functional recovery, as measured by positive, muscle-specific movement was positive in all animals. Distal nerve stumps demonstrated appreciable co-staining for neurofilament and S-100 indicating the presence of Schwann cells (See Figure 2).
- This preliminary work demonstrated the feasibility of establishing a 30mm complex branching nerve defect model in swine. Furthermore, this adds to the existing literature of the sural nerve's role in facial nerve reconstruction (see Figure 3 for sample sural nerve reconstruction image)
- After establishing the technique and confirming suitable outcomes, we refined our nerve testing technique (detailed below) to provide more precise and quantifiable functional recovery.

Figure 2a – S100 staining at 40x magnification:

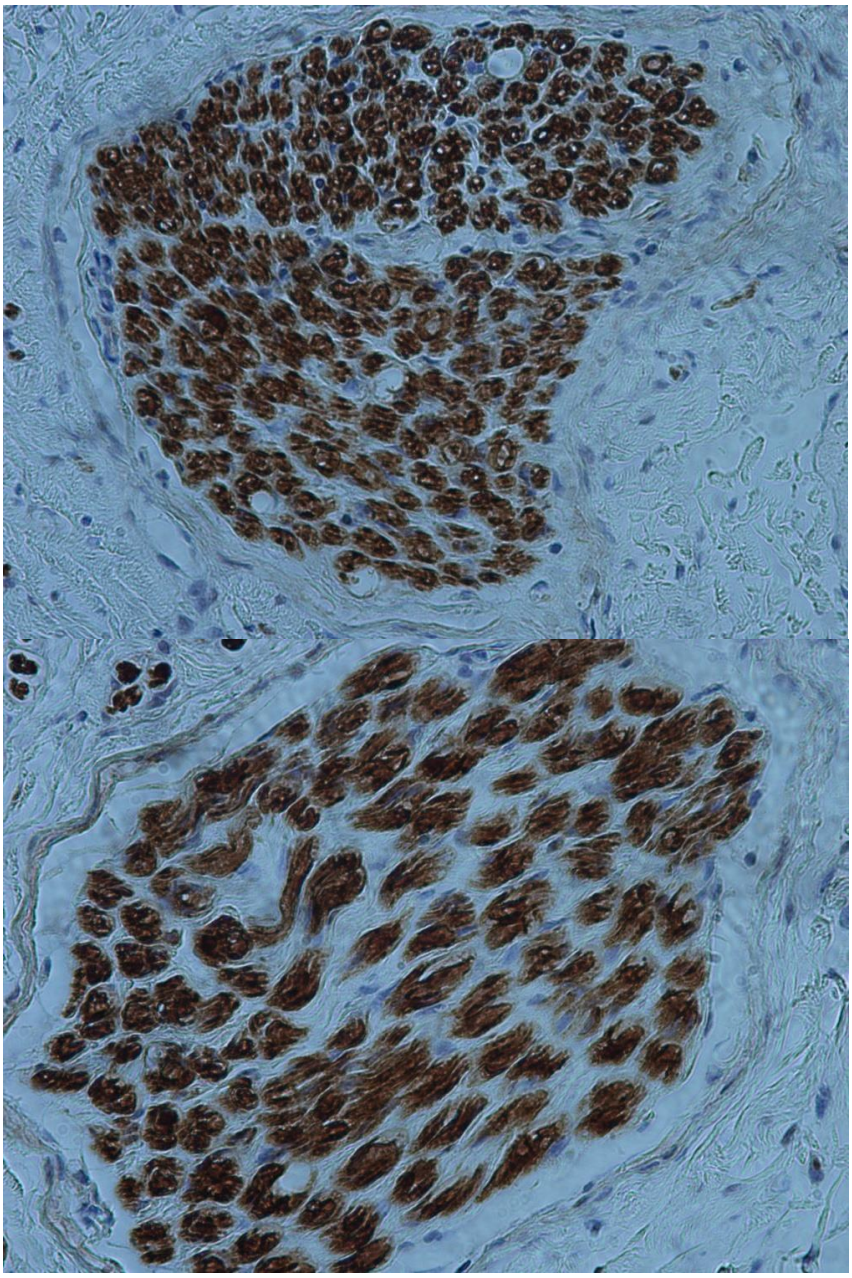


Figure 2b – NF staining at 4x magnification:

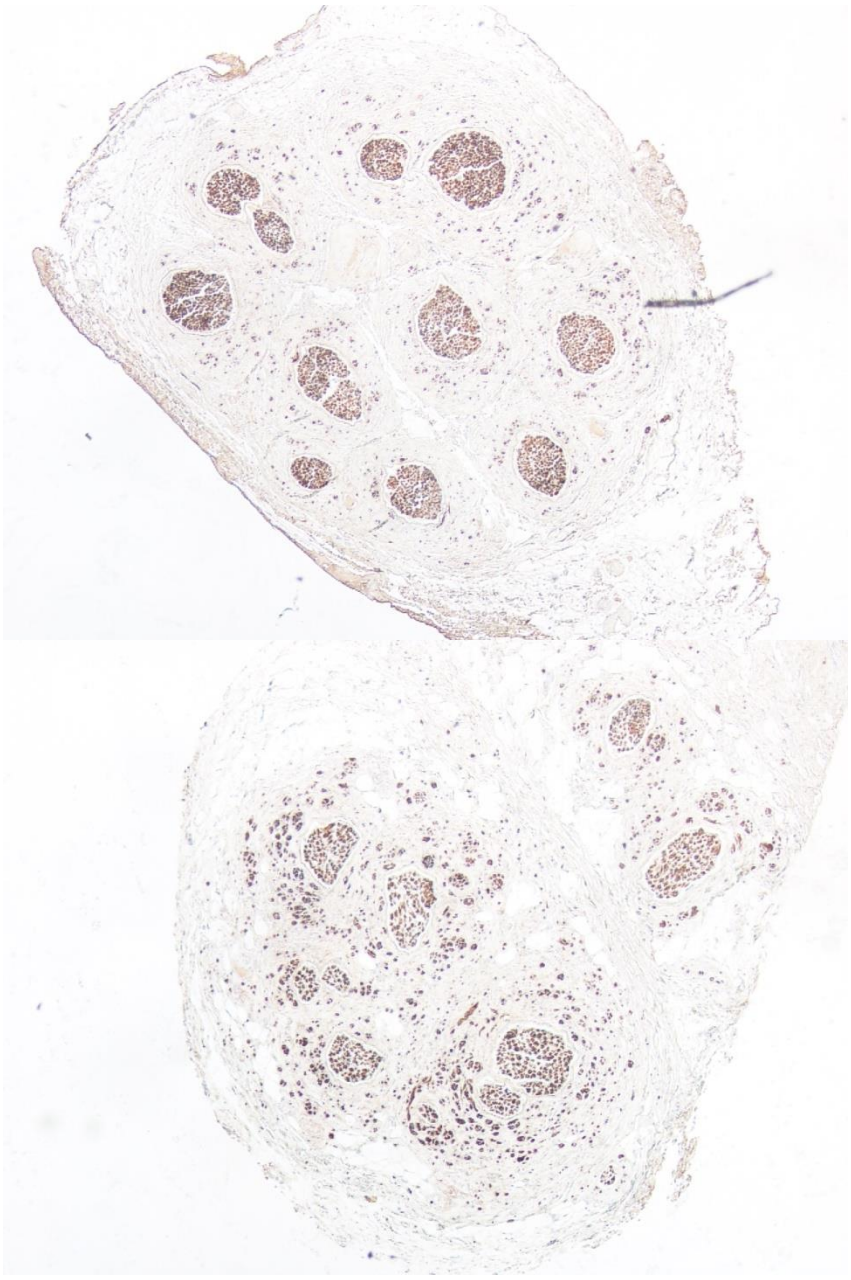
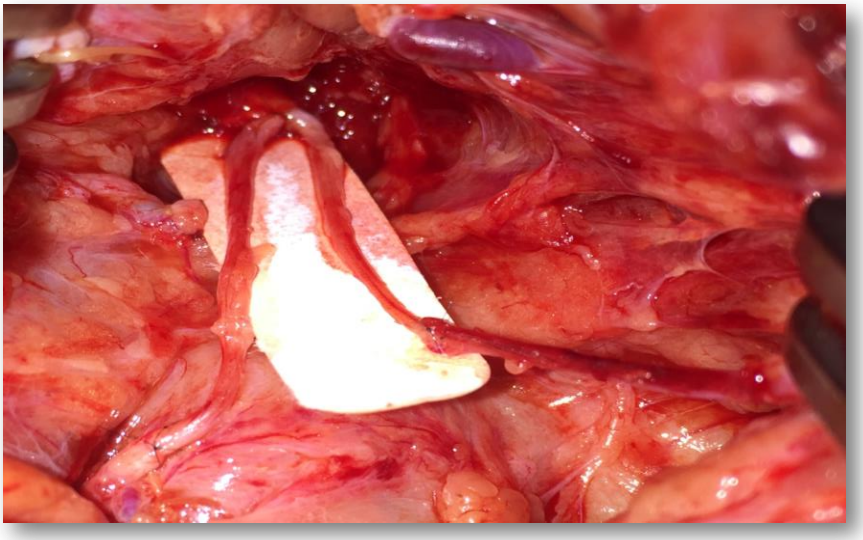


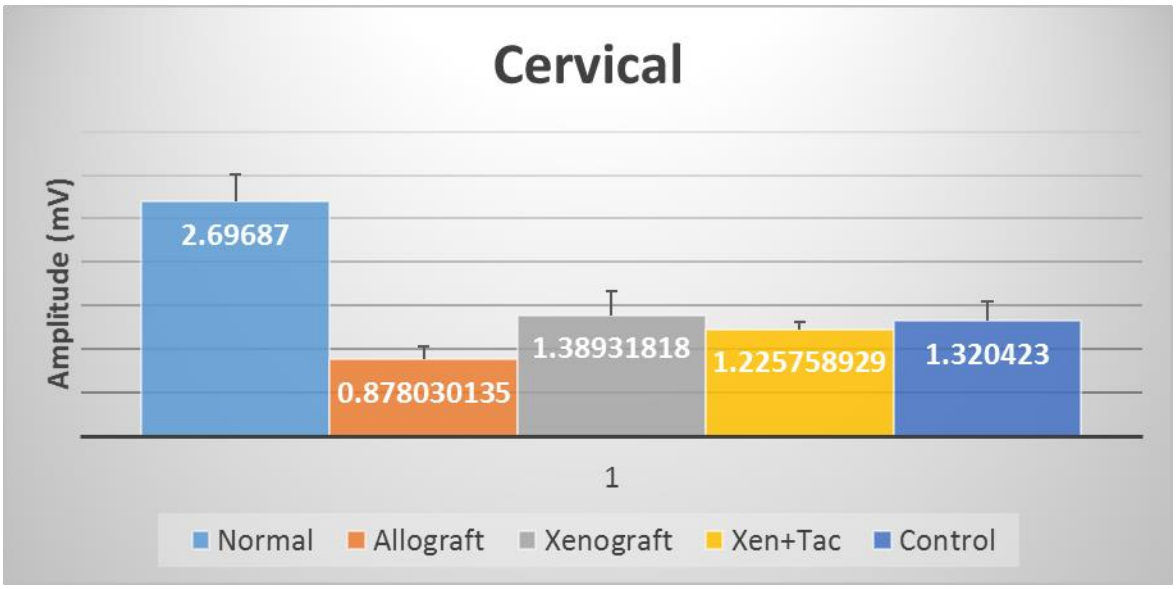
Figure 3. Sural nerve reconstruction

Control – Sural Nerve Repair



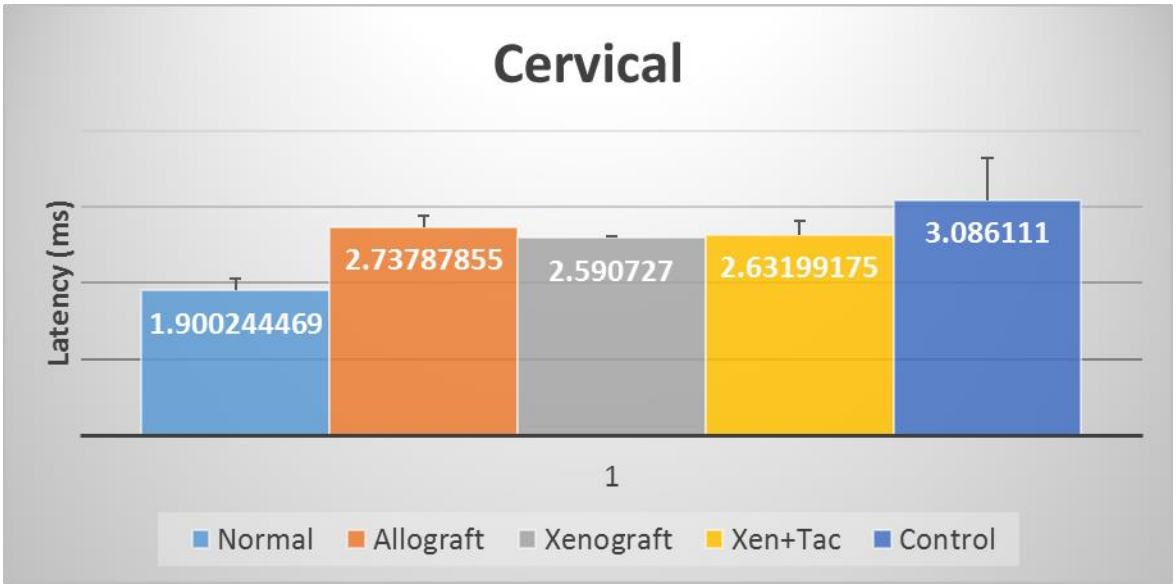
- 2) specific objectives;
  - Completion of all 18 allotted animal surgeries without mortalities.
  - Development of an intra-operative, direct nerve stimulation protocol to assess functional recovery (detailed in Methodology section below) as described in Henry FP et al, Improving electrophysiologic and histologic outcomes by photochemically sealing amnion to the peripheral nerve repair site. Surgery 2009 Mar;145(3):313-21.

- 3) significant results or key outcomes, including major findings, developments, or conclusions:
  - There were intrinsic functional differences between normal MMB (4.48±0.90 mV) and Cervical (2.70±0.99 mV) nerves ($p<0.0005$).
  - Amplitude (mV) and latency (ms) were utilized as functional outcomes in addition to the compound muscle action potential waveform, each of which is represented below:



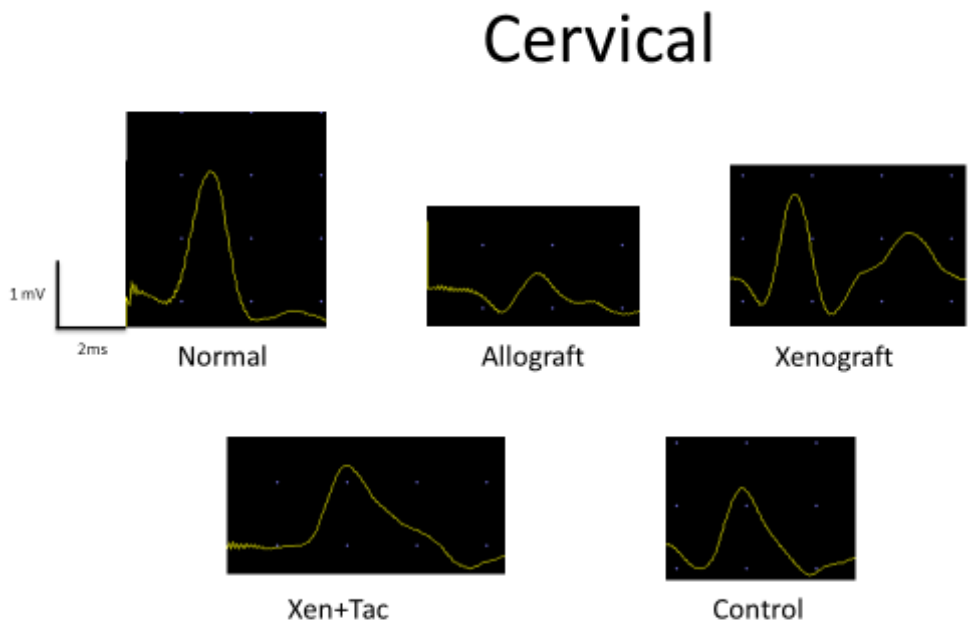
No significant differences between study groups.

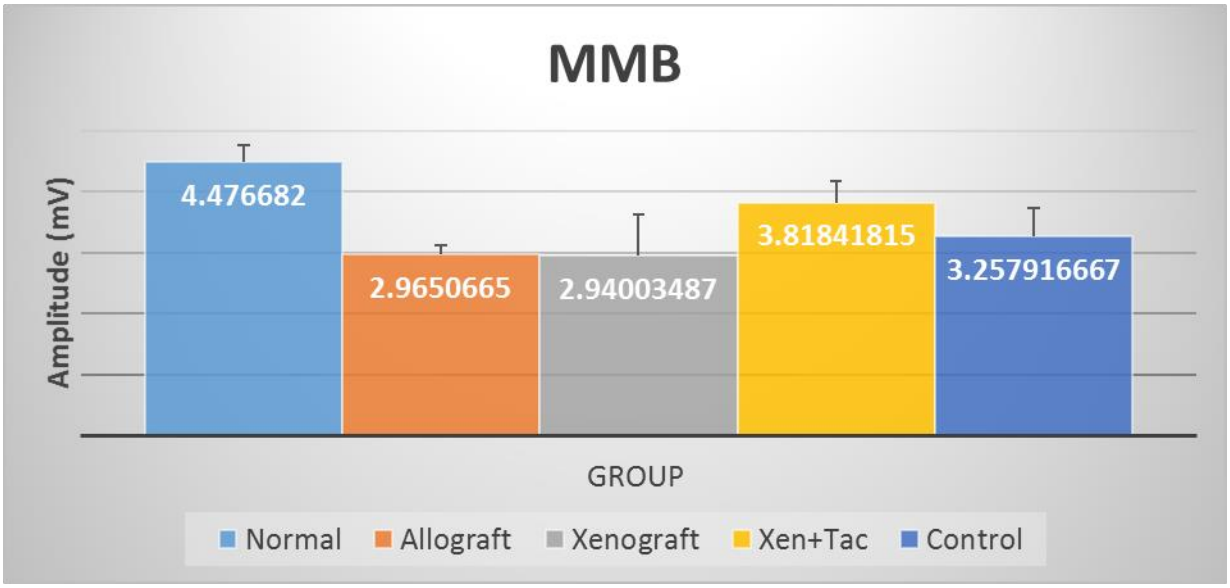
The differences in amplitude between normal nerves (2.70±0.99 mV) and allograft (0.88±0.31 mV) and Xen+Tac 1.23±0.16 mV) were significant (p=0.006 and p=0.03, respectively).



No significant differences between study groups.

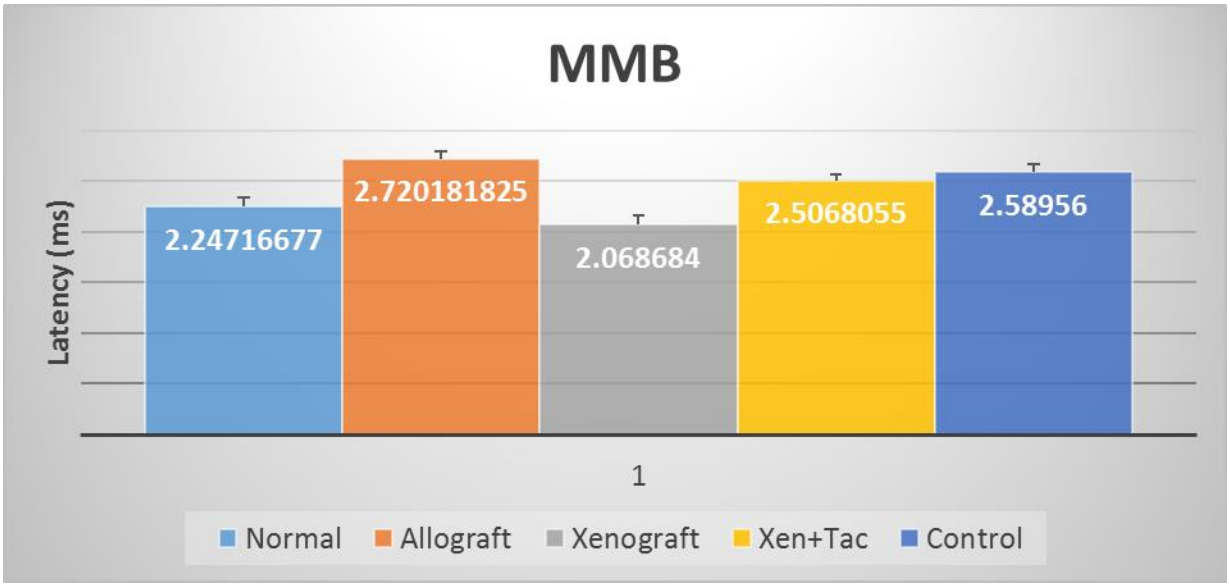
The difference in latency between normal nerves (1.90±0.47 ms) and autograft controls (3.09±0.96 ms) was significant. (p=0.019).


Cervical
1 mV
2ms
Normal
Allograft
Xenograft
Xen+Tac
Control


MMB
Amplitude (mV)
4.476682
2.9650665
2.94003487
3.81841815
3.257916667
GROUP
Normal
Allograft
Xenograft
Xen+Tac
Control

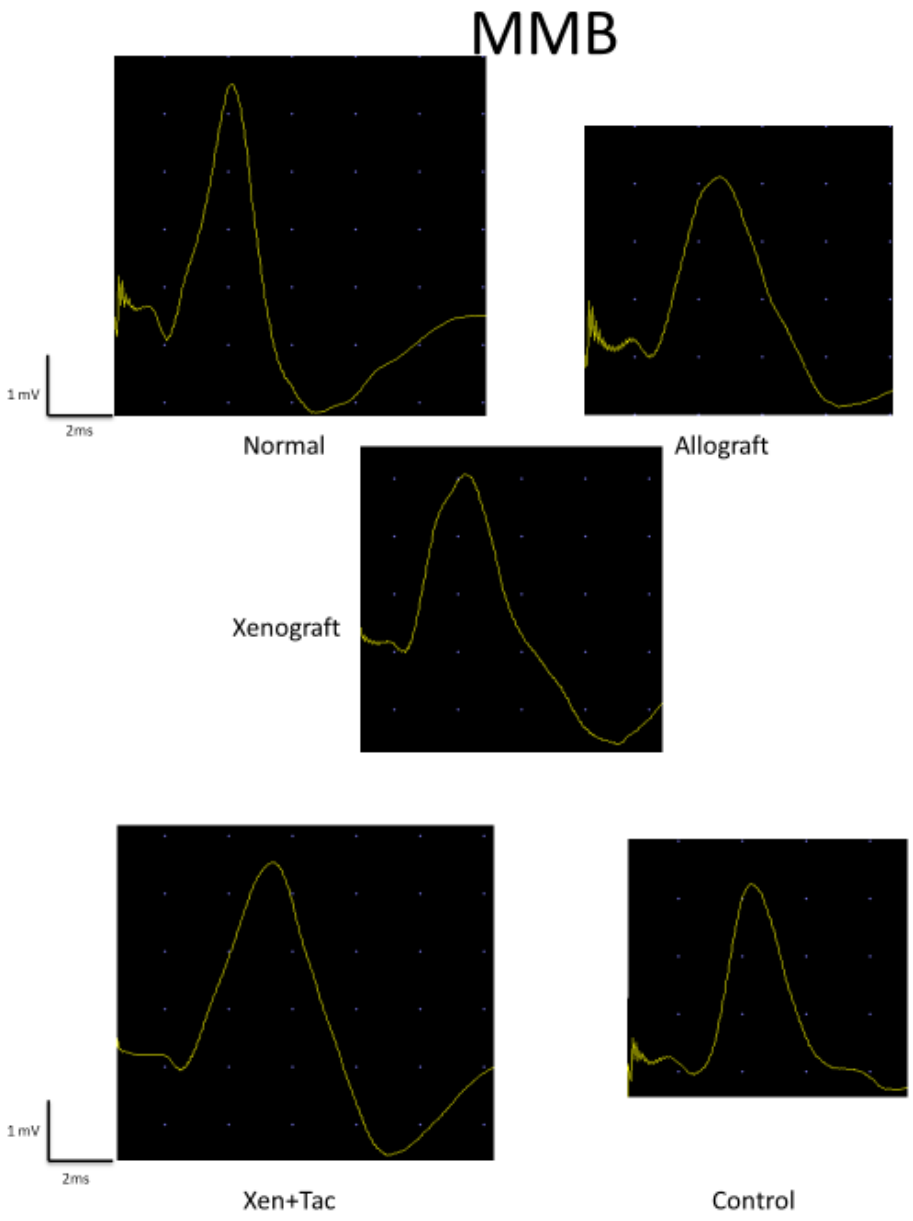
No significant differences between study groups.

The differences in amplitude between normal nerves (4.48±0.90 mV) and allograft (2.97±0.32 mV) were significant (p=0.045).



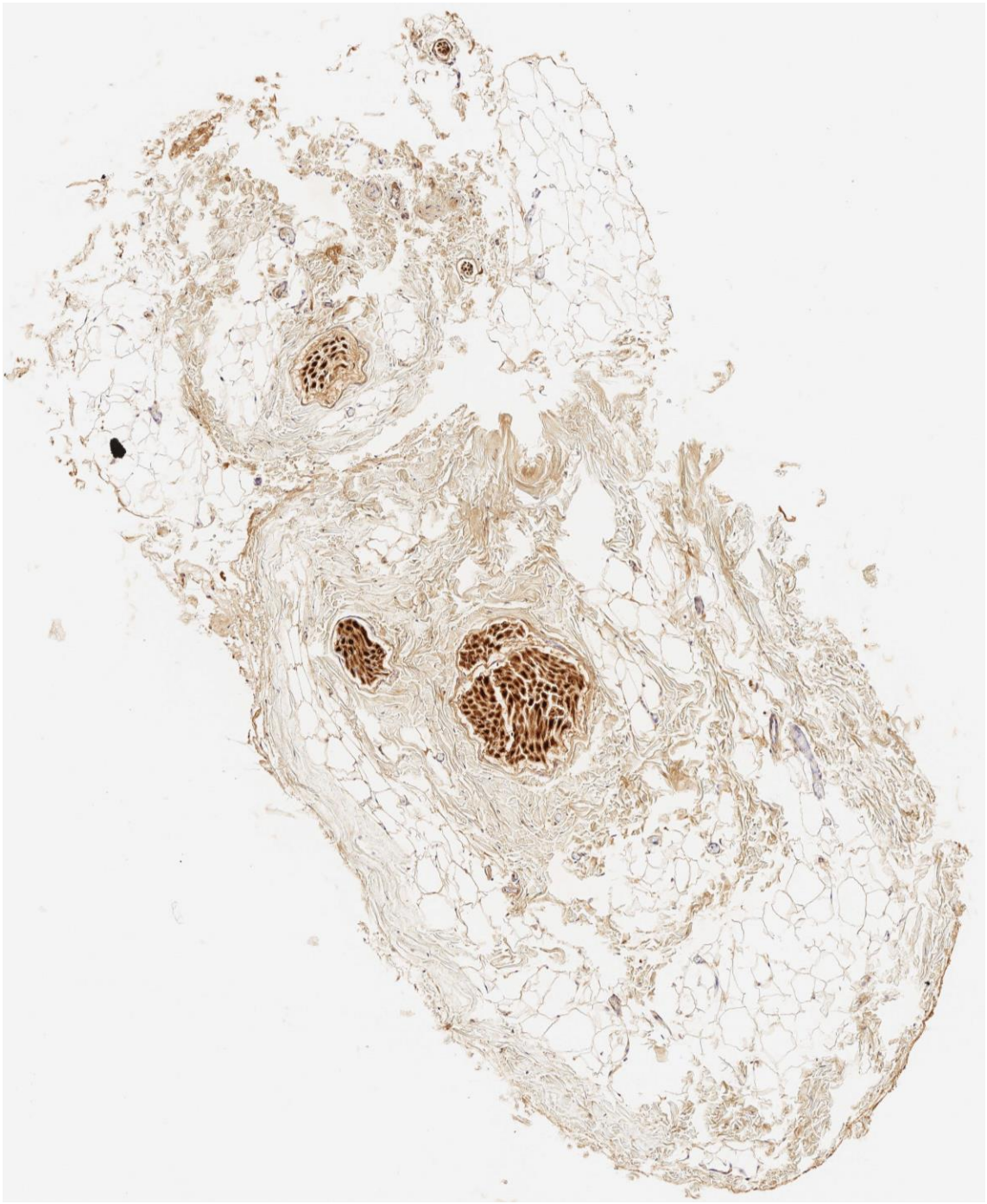
No significant differences between study groups.

The differences in latency between normal nerves (2.25±0.27 ms) and allografts (2.72±0.14 ms) and xenografts (2.07±0.13 ms) were significant. (p=0.015 and p=0.025, respectively).

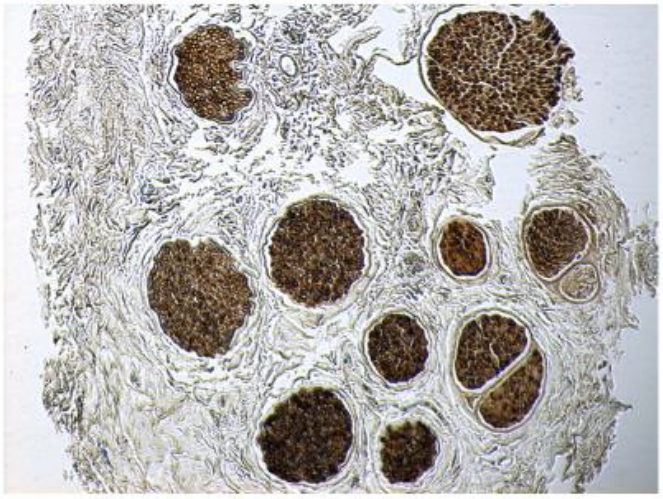


Gross histological outcomes are presented below:

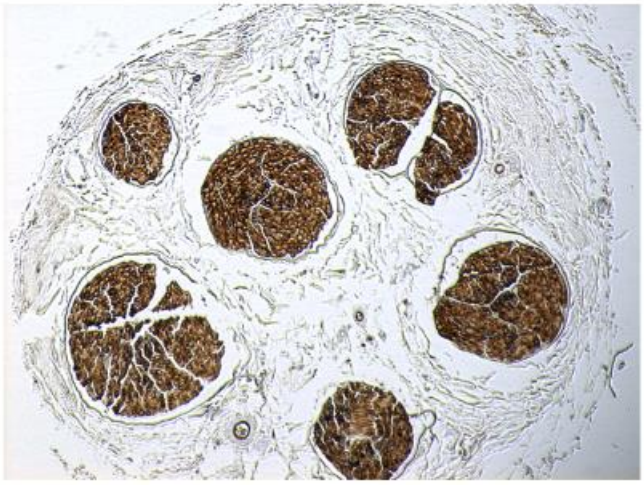
<u>Normal nerves</u> –



Cervical nerve

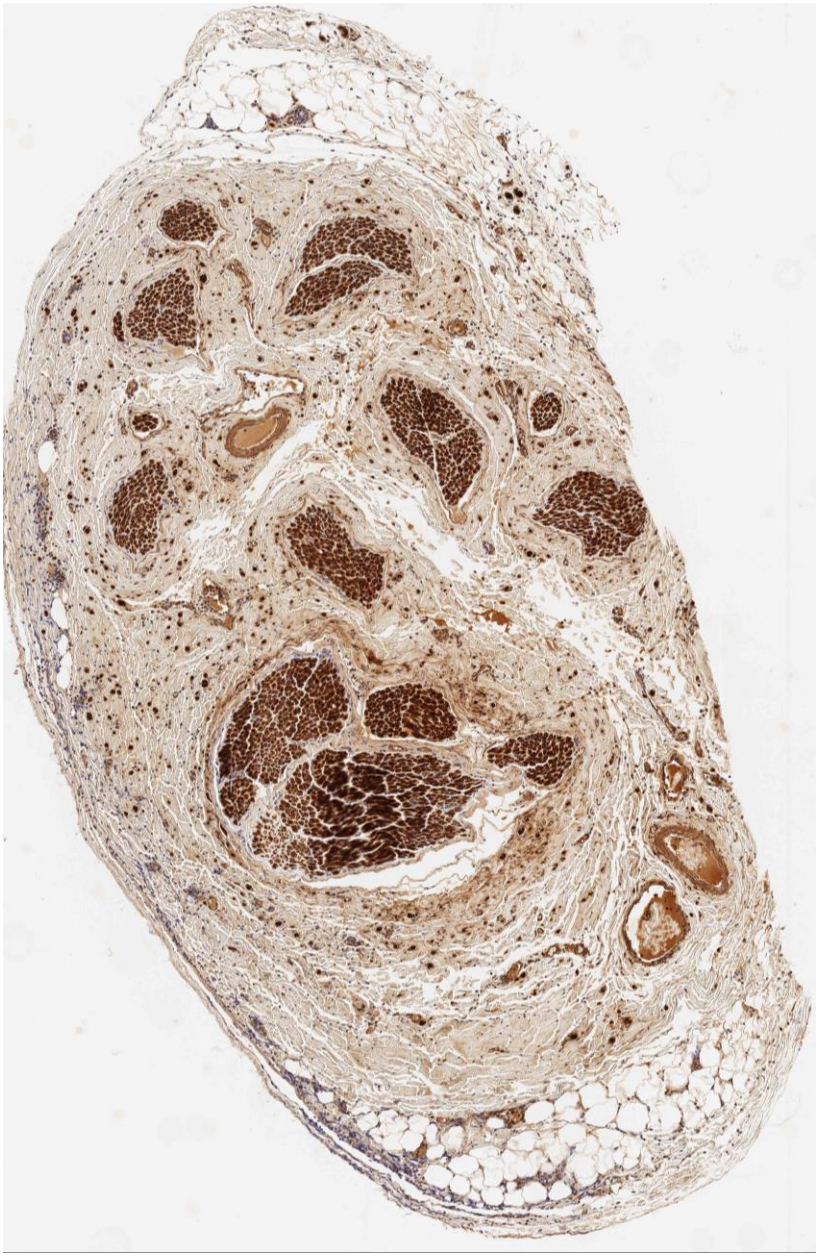


MMB

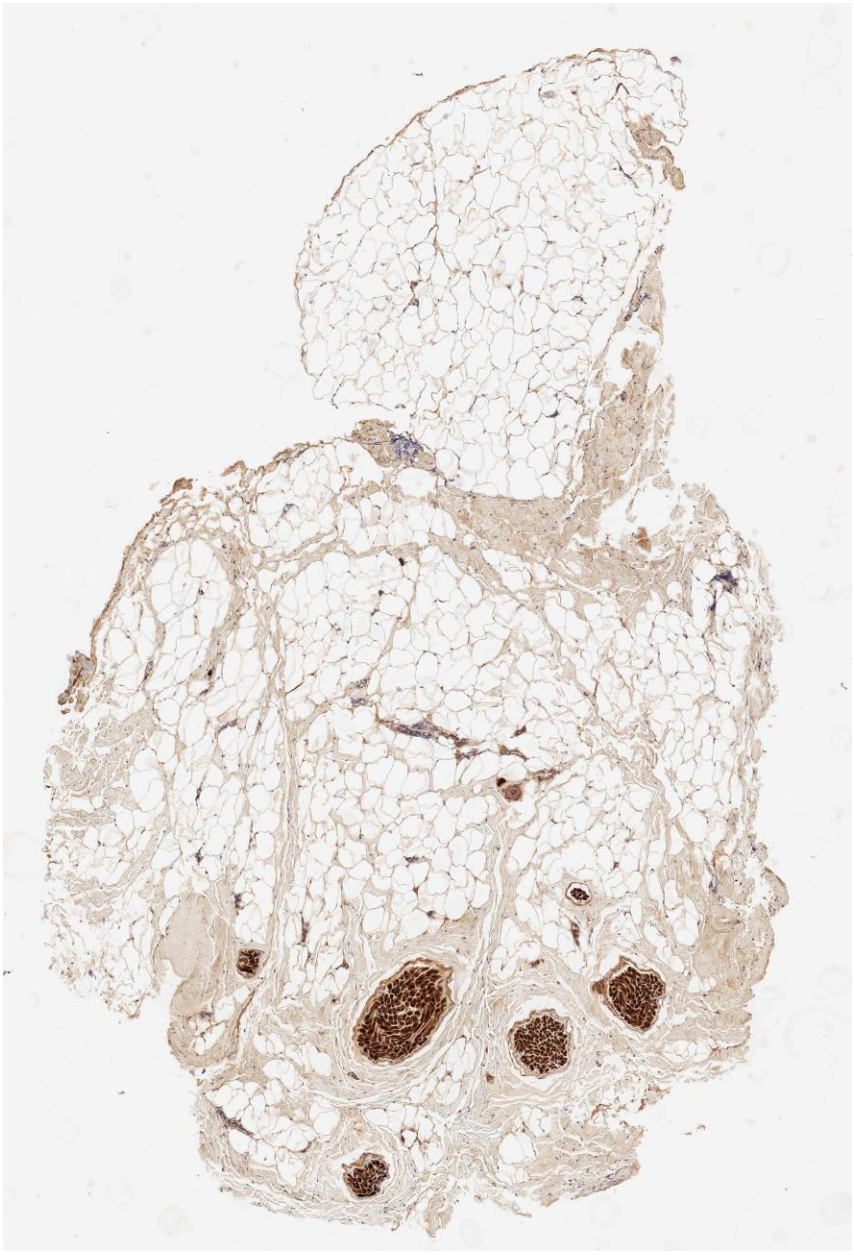


Sural Nerve

Control – autograft:

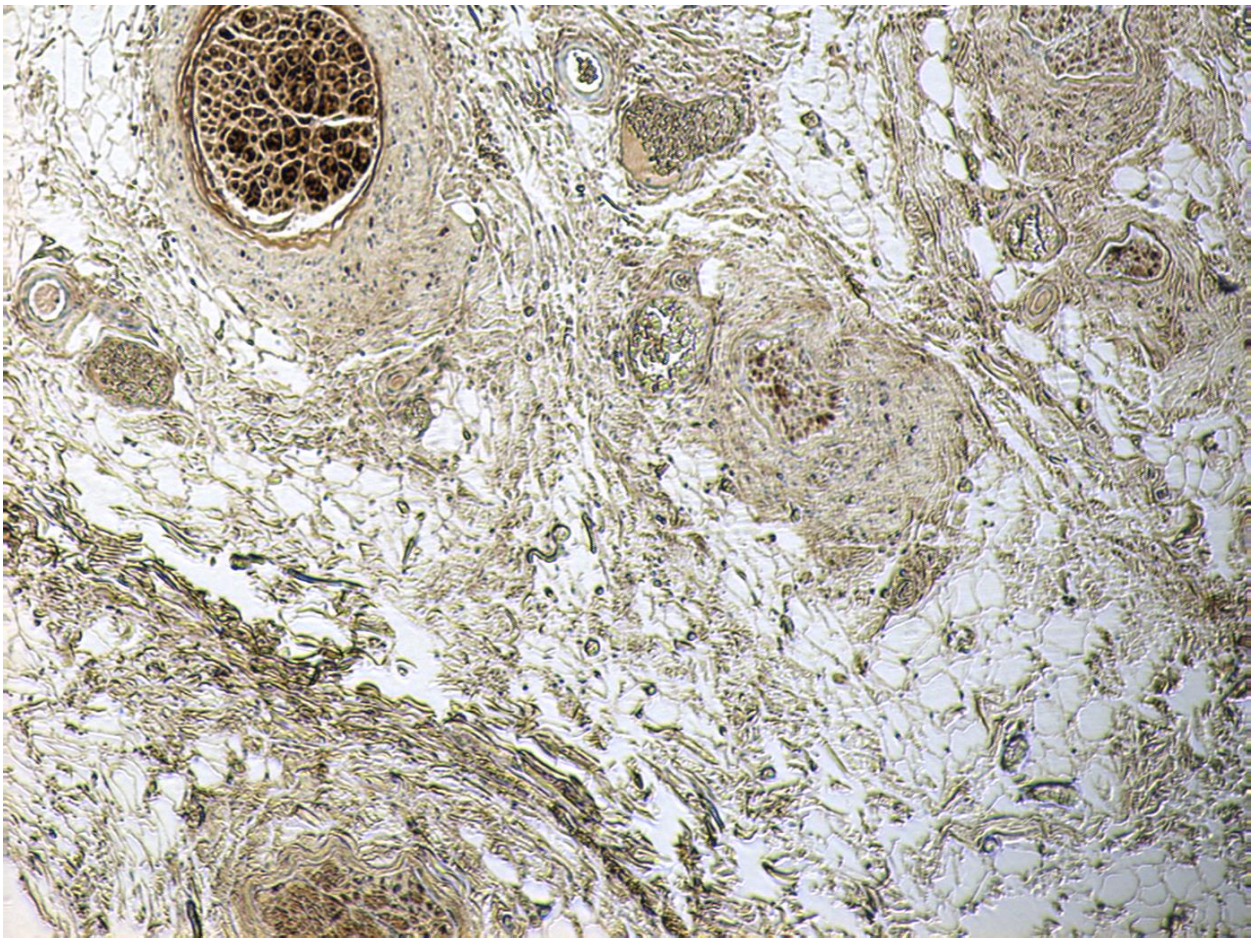


MMB (autograft)

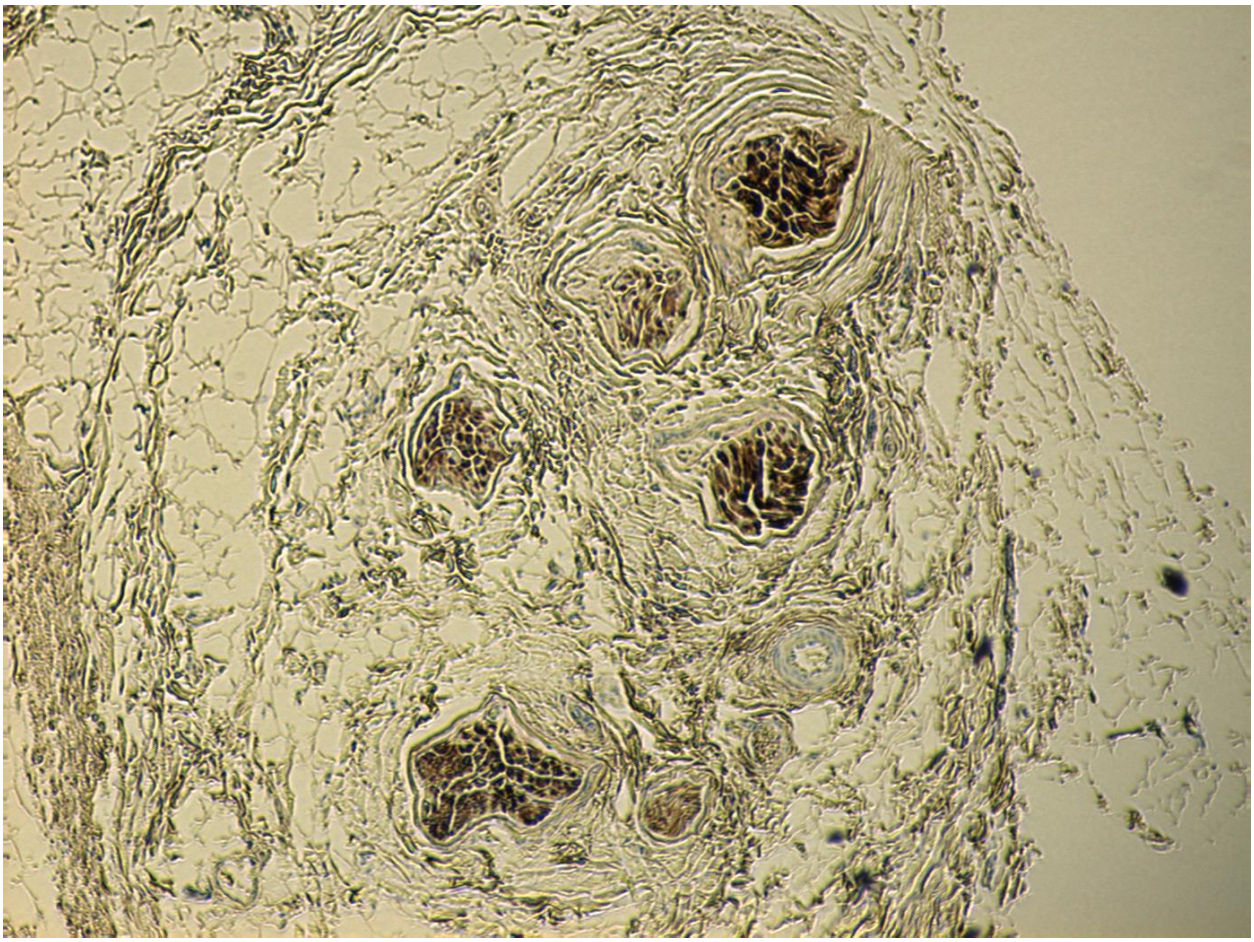


Cervical (autograft)

Allograft:

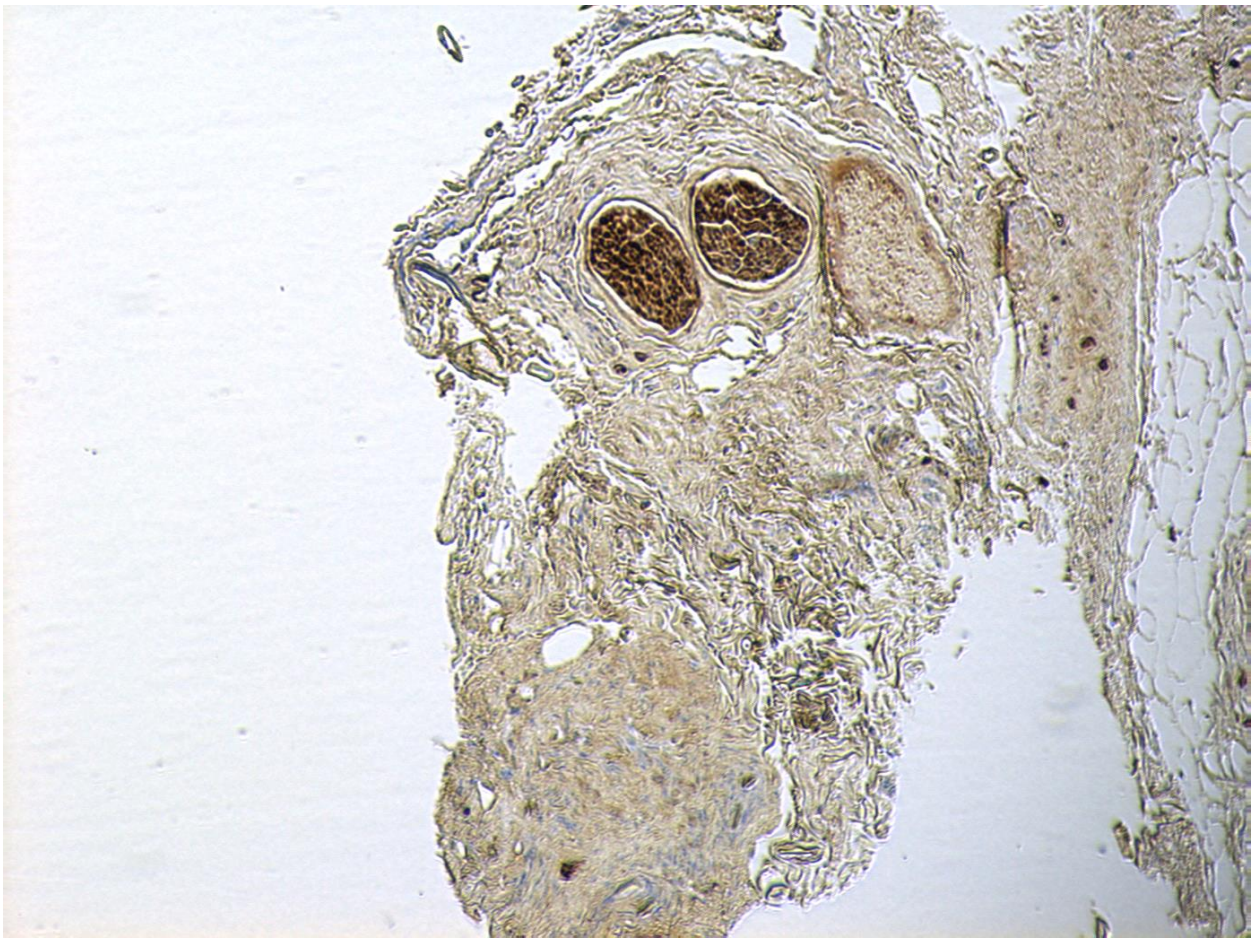


Cervical nerve allograft

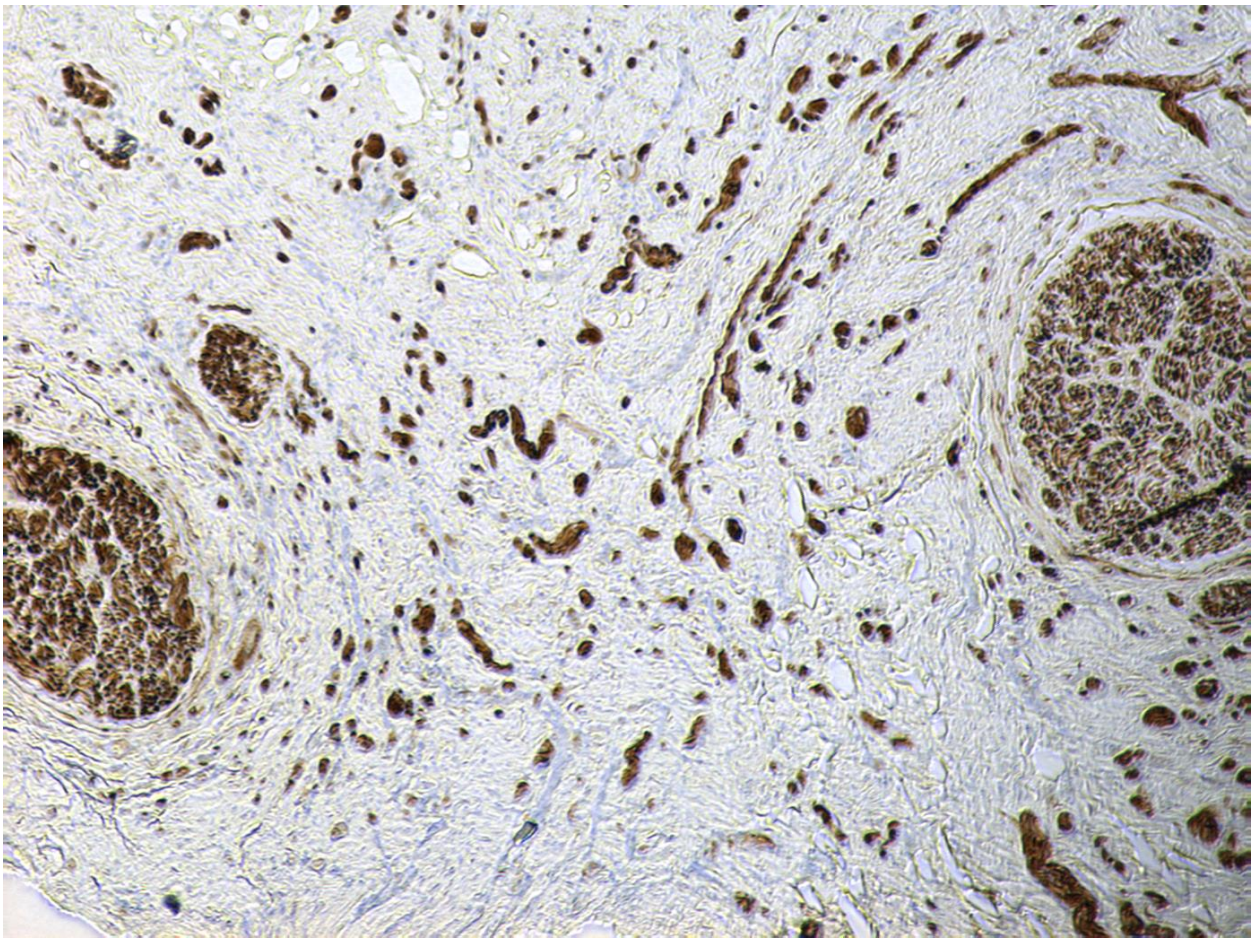


MMB nerve allograft

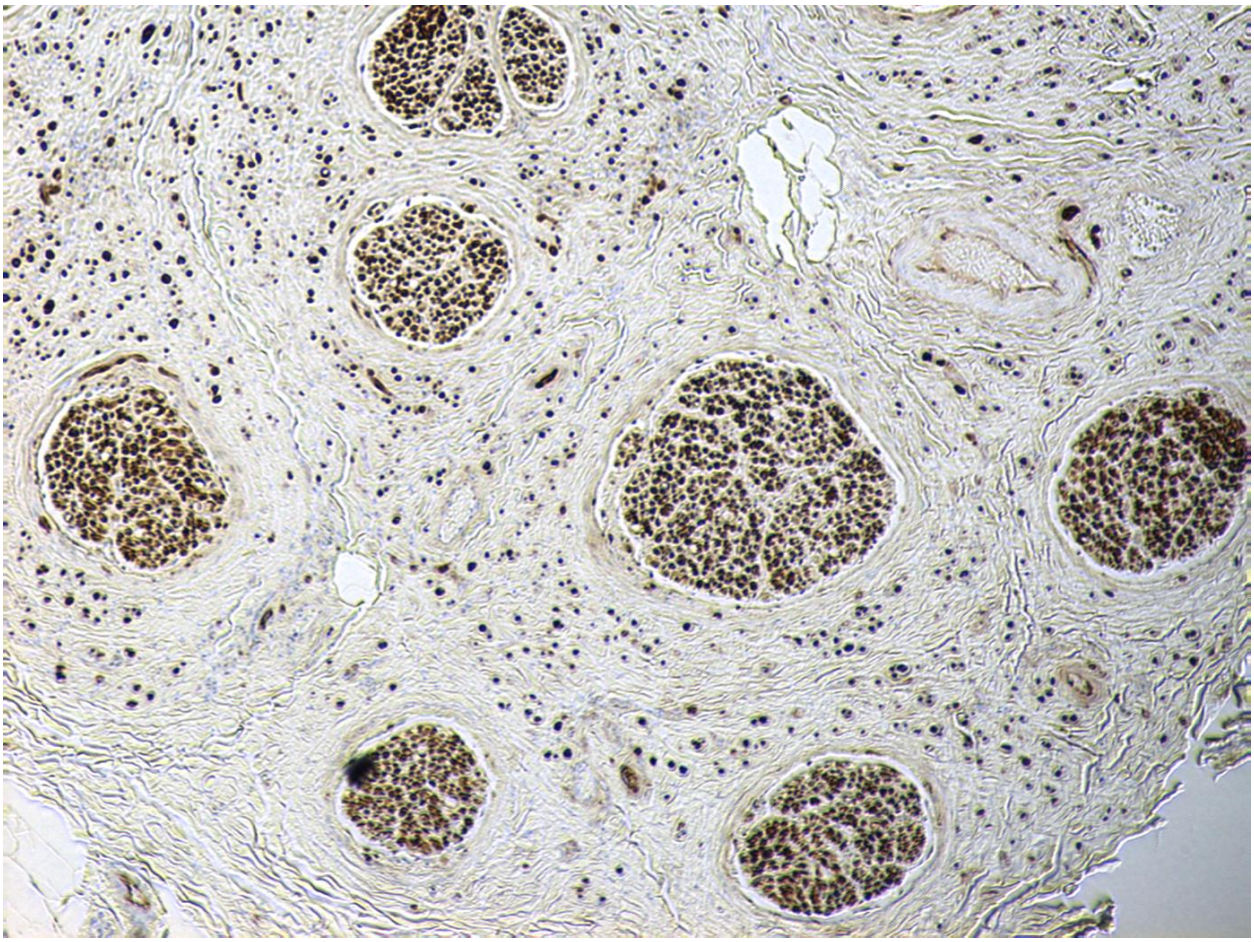
Xenograft:



Cervical nerve Xenograft

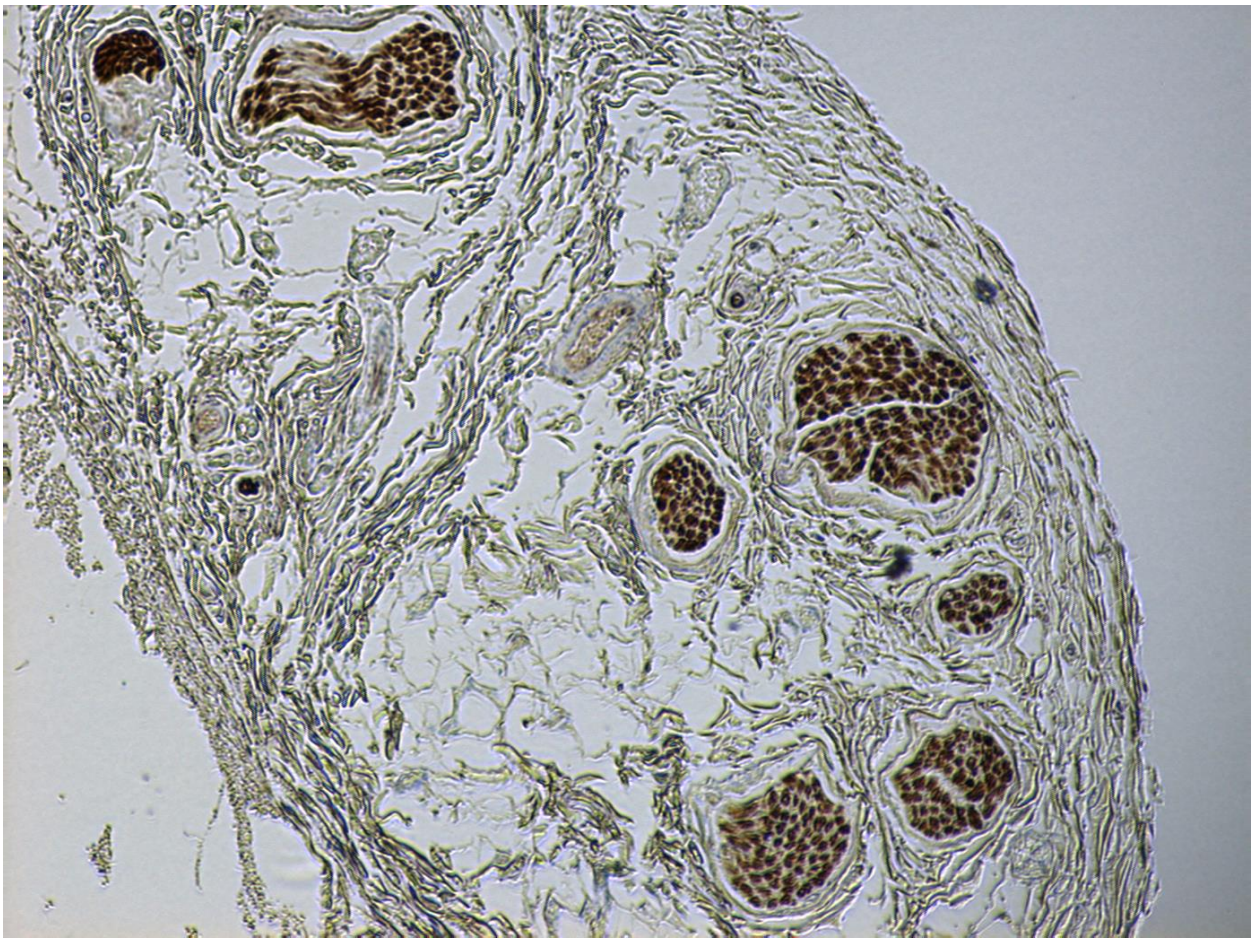


Cervical nerve Xenograft

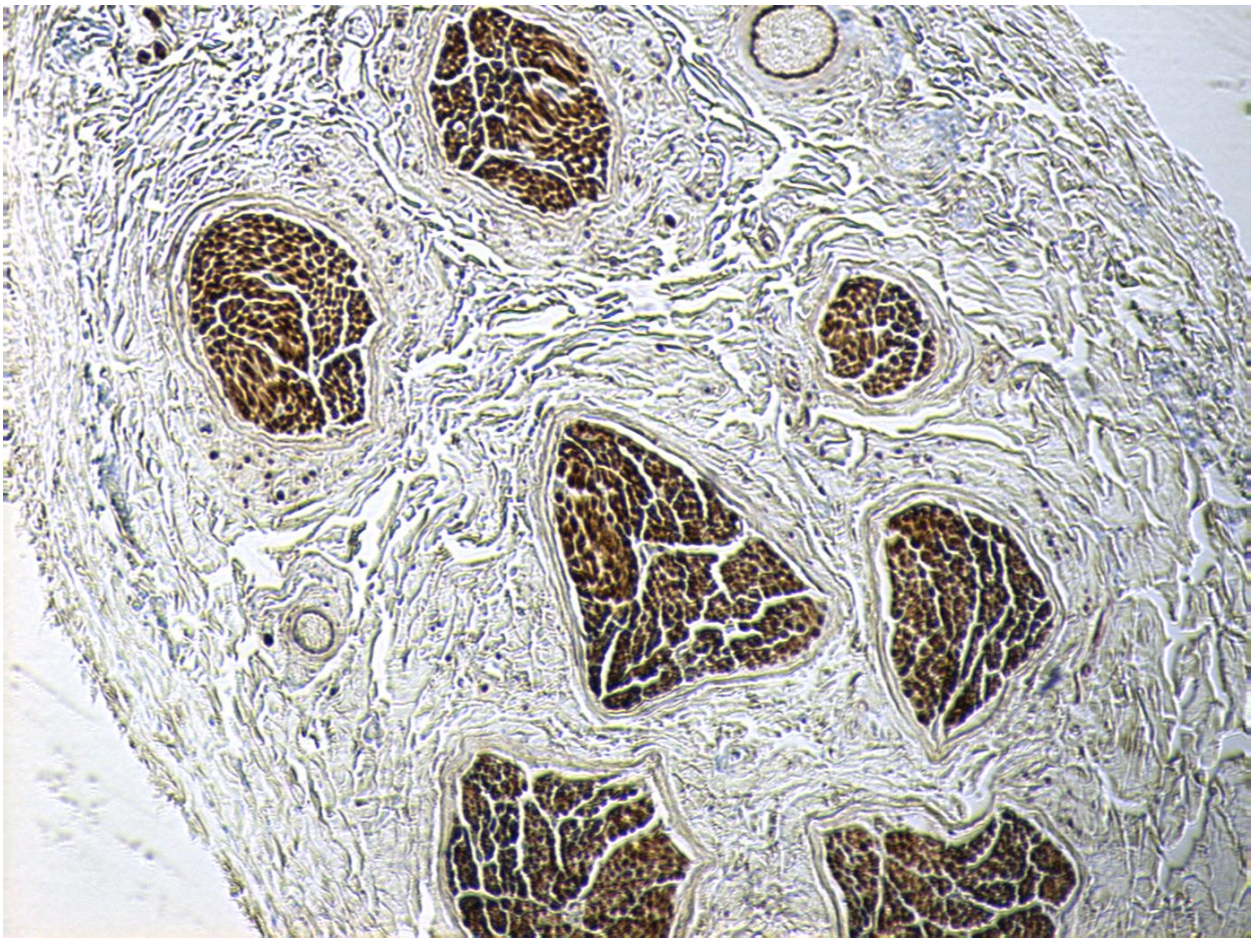


MMB nerve Xenograft

Xenograft + Tacrolimus:



Cervical nerve Xenograft+Tacrolimus



MMB nerve Xenograft+Tacrolimus

Data related to study animals receiving Tacrolimus:

Tacrolimus (Accord Healthcare, Durham, NC, USA) administration was administered orally as a one-time dose (0.3mg/kg BW) pre-operatively and continued immediately after xenograft coaptation as a twice daily oral administration and continued for the entire duration of study (i.e., 24 weeks). Initial dosing was given at 0.15mg/kg BW twice daily with resulting trough levels <4 ng/mL. Dosing was adjusted (ranging from 0.35-0.45mg/kg BW) accordingly to reach goal trough levels. To facilitate oral medication administration, the tacrolimus capsules were hidden in Greenies Pill Pockets (The Nutro Company, Franklin, TN). Goal whole blood trough levels were set to 4-8 ng/mL in order to evaluate the role of low-dose Tacrolimus on nerve regeneration using a human-derived processed nerve graft (i.e., xenograft) while minimizing the side effects of chronic Tacrolimus and drug-related toxicity.

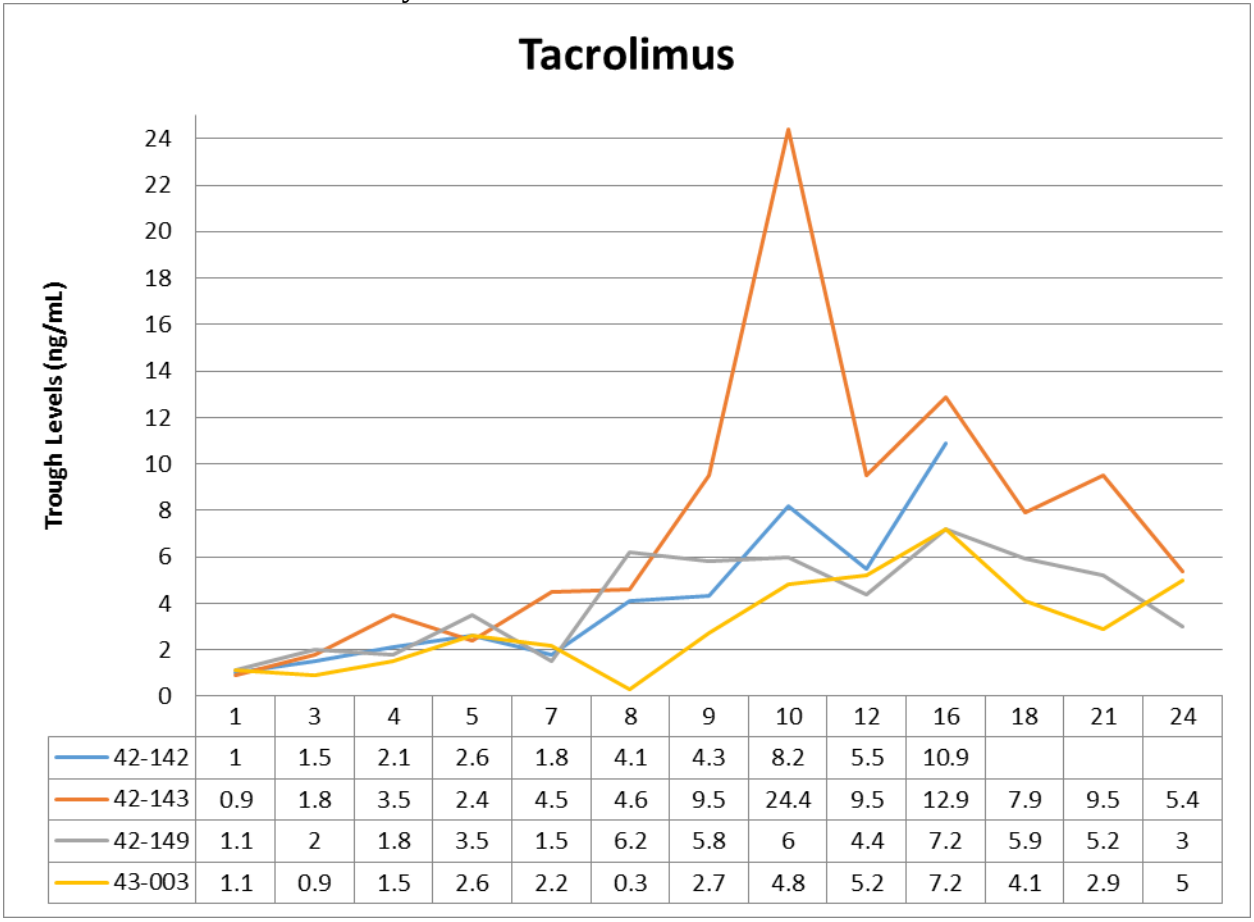
At the study end point, there was no difference in the average weight for the Tacrolimus group 60.25±6.2kg and the rest of the cohort 58.5±5.0kg (p=0.60). Therefore, the Tacrolimus group average weight was appropriate and represents normal weight gain despite the administration of oral Tacrolimus which can often be marked by anorexia and metabolic/GI disturbances.

One animal in the Xenograft + TACROLIMUS group (# 42-142) developed severe anorexia and subsequent weight loss about 16 weeks after surgery and therapy that required stopping the medication as we could not force her to take the medication orally. Her weight decreased from 49.1 kg to 44.2 kg in 2 months. After discussions with the veterinary staff, she was not empirically treated with antibiotics and rather managed expectantly off of the Tacrolimus. She eventually recovered her weight (eventually gained back 7 kg to a terminal weight of 51.3 kg) and was eating normally without signs of any infection. The terminal weight of 51.3 kg was approximately 9 kg less than the group average. Functional and histologic recovery were unaffected.

Table 1: Weight for Xenograft+Tacrolimus group:

#:	**Pre-op Weight(kg)**	**Post-Op Weight (kg)**
42-142	30.8	51.3
42-143	29.8	61.1
42-149	30.7	64.8
43-003	31.9	63.8
Group Average:	30.8	60.25
SD:	0.860233	6.167928

Table 2 – Tacrolimus levels by animal and week:



	1	3	4	5	7	8	9	10	12	16	18	21	24
42-142	1	1.5	2.1	2.6	1.8	4.1	4.3	8.2	5.5	10.9			
42-143	0.9	1.8	3.5	2.4	4.5	4.6	9.5	24.4	9.5	12.9	7.9	9.5	5.4
42-149	1.1	2	1.8	3.5	1.5	6.2	5.8	6	4.4	7.2	5.9	5.2	3
43-003	1.1	0.9	1.5	2.6	2.2	0.3	2.7	4.8	5.2	7.2	4.1	2.9	5

Table 3 – Tacrolimus levels (mean±SD)

	Tacrolimus trough levels (ng/mL)
Group:	**5.7 ± 4.9**
42-142:	4.2 ± 3.2
42-143	7.4 ± 6.2
42-149	4.1 ± 2.1
43-003	3.1 ± 2.0

- **Goals not met** – proper preparation/preservation and staining to allow for histomorphometric analysis.
  - This task was outsourced to the Harvard Medical School

Electron Microscopy (EM) laboratory who has extensive expertise in preparation of tissues for EM. Preparation for histomorphometry is tedious and problems with sectioning of specimens were related to the direction in which they were sectioned and staff unaware of how to best deliver the cross-sections that would yield the most accurate representation of that nerve or the staining was too dark. Certain aspects of the process improved with time and repeat sectioning, but overall proved to be costly and precluded an accurate histomorphometric analysis or axon counts.

- We are currently working to analyze the number of fascicles (nerve bundles and their area) as a surrogate marker of histological recovery and quantitative histologic assessment (Zhu Y et al, Vascularized versus Nonvascularized Facial Nerve Grafts Using a New Rabbit Model. Plast Reconstr Surg. 2015 Feb;135(2):331e-9e.).

- **Methodology:**
- **Animal Care**
  - Animal studies were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* under experimental protocols approved by the Animal Studies Committee at Harvard Medical School. Animals were housed in a central animal care facility and given access to a specially manufactured chow (Laboratory Mini-Pig Grower Diet 5081, LabDiet, St. Louis, MO), fruits, vegetables and water ad libitum. Postoperatively, all animals were evaluated twice daily for the first four days and then daily with additional examinations performed as needed. Animals receiving Tacrolimus (Accord Healthcare, Durham, NC, USA) were monitored twice daily and weighed during serial Tacrolimus trough level measurements.
- **Surgical Procedures**
  - Dissections were performed through a retromandibular incision one fingerbreadth caudad to the angle of the mandible with a submandibular extension. The platysma was then exposed by way of skin flaps and split to expose the superficial layer of the deep cervical fascia. The dissection was carried down deep to the parotid gland where a notable styloid process and stylohyoid tendon served as reference points for all dissections. Maxillary veins and tributaries were ligated as needed for greater exposure. The inferior division of the facial nerve was identified, isolated and sharply transected at a point 5mm proximal to the branching point of the marginal mandibular and cervical nerve branches. Approximately 25mm distal to the transection, the marginal mandibular and cervical nerves were isolated and sharply transected to create a 30mm nerve gap.

The deep cervical fascia and the platysma were reapproximated using 3-0 Vicryl suture (Ethicon, Inc. Somerville, N.J.) and skin closed in layers using 4-0 Monocryl suture (Ethicon, Inc. Somervile, N.J.). Postoperatively, animals were recovered per standard protocol in the animal care facility.

- **Experimental Design**
  - Thirteen Yucatan miniature swine (Sinclair BioResources, St. Louis, MO) weighing 25-30kg were randomized into four experimental groups containing two to four animals per group. All animals underwent facial dissection and facial nerve transection as described above. One group of animals (autograft group, *n*=3) underwent sural nerve harvest for autografting as the method of nerve gap repair. Another group underwent allograft nerve repair using a species-specific processed facial nerve allograft (allograft group, *n*=4). Another experimental group underwent xenograft nerve repair using a processed, human-derived nerve graft (xenograft group, *n* =2). Similarly, the final experimental group underwent xenograft nerve repair using a processed, human-derived nerve graft with the administration of Tacrolimus throughout the entirety of the study (xenograft+Tacrolimus group, *n* =4).
- **Immunosuppressive medication and Blood analyses:**
  - To avoid chronic indwelling venous catheters in our long-term large animal model, we initially followed Jensen-Waern and colleagues' orally-administered immunosuppressive protocol for growing swine. In this study, the authors used tacrolimus and mycophenolic acid with eligible whole blood trough value for tacrolimus of 5-15 ng/mL where mycophenolic acid was given at a fixed dose of 500mg twice daily (Jensen-Waern M, Kruse R, Lundgren T. Oral immunosuppressive medication for growing pigs in transplantation studies. Laboratory animals 2012;46:148-51). Therefore, in our study, Tacrolimus administration was administered orally as a one-time dose (0.3mg/kg BW) pre-operatively and continued immediately after xenograft coaptation as a twice daily oral administration and continued for the entire duration of study (i.e., 24 weeks). Initial dosing was given at 0.15mg/kg BW twice daily with resulting trough levels <4 ng/mL. Dosing was adjusted (ranging from 0.35-0.45mg/kg BW) accordingly to reach goal trough levels. To facilitate oral medication administration, the tacrolimus capsules were hidden in Greenies Pill Pockets (The Nutro Company, Franklin, TN). Goal whole blood trough levels were set to 4-8 ng/mL in order to evaluate the role of low-dose Tacrolimus on nerve regeneration using a human-derived processed nerve graft (i.e., xenograft) while minimizing the side effects of chronic tacrolimus and drug-related toxicity.
  - EDTA-preserved blood was analysed for tacrolimus whole blood trough levels by way of an immunoassay (ARCHITECT Tacrolimus assay; Abbott Laboratories, Abbott Park, IL). Blood samples were collected

under sedation using intramuscular Telazol (Pfizer, Florham Park, NJ) 4.4mg/kg BW and Atropine (Med-Pharmex, Pomona, CA) 0.05mg/kg BW. Whole blood trough levels were obtained via venipuncture using the marginal ear vein and trough-level monitoring performed 12 h after the last dose.

- **Electrophysiologic Assessment:**
  - Electrophysiologic measurements were performed before surgery and postoperatively at 24 weeks after surgery (i.e., defined end point). Measurements were taken using an UltraPro S100 EMG system (Natus Medical Inc, Middleton, WI) from both experimental (left) and control (right) nerves. Disposable, adhesive surface recording electrodes (part #019-415200, Natus Medical Inc, Middleton, WI) were placed over the depressor labii inferioris muscle and the sternocephalicus muscle, for the marginal mandibular nerve and cervical nerve, respectively. Using a TECA Monopolar Needle (28 G × 1.00 in; recording area 0.28mm^2; part #902-DMF25-TP, Natus Medical Inc, Middleton, WI) direct nerve stimulation was performed on the marginal mandibular nerve and cervical nerve 5mm below the main branching point. Using stimulus duration of 0.02 ms, stimulus intensity was increased gradually until a supramaximal compound muscle action potential was obtained. At least 10 consecutive traces were averaged.
  - Compound muscle action potential (CMAP) amplitude was measured as the difference in voltage (mV) from baseline to peak. CMAP latency (ms) was measured from stimulus to negative peak onset. Lastly, CMAP duration (ms) was measured as the time between the rising and declining curves.
- **Tissue preparation for histomorphometry:**
  - Repaired facial nerves (recipient/graft complexes) were harvested and were postfixed for at least 2 hours at room temperature in 2.5% Glutaraldehyde 2% Paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.4), washed in 0.1M cacodylate buffer and postfixed with 1% Osmium tetroxide (OsO4)/1.5% Potassium ferrocyanide(KFeCN6) for 1 hour, washed in water 3x and incubated in 1% aqueous uranyl acetate for 1 hour followed by 2 washes in water and subsequent dehydration in grades of alcohol (10min each; 50%, 70%, 90%, 2x10min 100%). The samples were then placed in propyleneoxide for 1 hr and infiltrated overnight in a 1:1 mixture of propyleneoxide and TAAB Epon (Marivac Canada Inc. St. Laurent, Canada). The following day the samples were embedded in TAAB Epon and polymerized at 60 degrees C for 48 hrs.
  - Cross-section (1µm) were cut on a Reichert Ultracut-S microtome from nerve distal to the site of coaptation using an ultramicrotome, stained with toluidine blue, and evaluated under light microscopy for preservation of nerve architecture, quality and quantity of regenerated nerve fibers, and extent of myelination.

- **Statistical Analysis:**
  - Data were organized via summary statistics using means and percentages as appropriate with corresponding standard deviation (SD) or as frequency distributions for continuous and categorical variables, respectively. One-way analysis of variance was performed to evaluate the differences between study groups with Bonferroni correction performed for multiple testing. All statistical analyses were conducted using STATA version 14 (STATA Corp., College Station, TX.). All statistical tests were two sided and significance was indicated by P-values less than 0.05.

- **What opportunities for training and professional development has the project provided?**
  - Professional development activities included participation and presentation of preliminary findings in the 2017 American Society of Peripheral Nerve (ASPN) by postdoctoral fellow, Mario Aycart, MD.
- **How were the results disseminated to communities of interest?**
  - Nothing to Report.
- **What do you plan to do during the next reporting period to accomplish the goals?**
  - Nothing to Report

4. IMPACT:

a. **What was the impact on the development of the principal discipline(s) of the project?**

i. Having a branched nerve allograft that allows for the reduction of the number of surgical sites while theoretically reducing the operative time (not formally measured or assessed in this study) and provides functional and histologic nerve recovery when compared to the current standard of care in autograft nerve repair would revolutionize nerve surgery. This would be a logical extension of the current AxoGen product of a straight processed nerve allograft.

ii. Another important aspect of this research, are the results from human nerve xenografts with and without Tacrolimus successfully demonstrating nerve regeneration in this porcine model. This aspect warrants additional research.

b. **What was the impact on other disciplines?**

i. "Nothing to Report."

c. **What was the impact on technology transfer?**

i. "Nothing to Report."

d. **What was the impact on society beyond science and technology?**

i. Nothing to Report

5. CHANGES/PROBLEMS:

a. **Changes in approach and reasons for change**

i. As previously reported, in the original proposal we were tasked with

performing monthly nerve conduction studies to track the progress of functional recovery. However, due to challenging anatomy and reliability of results using the aforementioned five model development control animals, we trialed and formally switched to the direct intra-operative nerve stimulation for our electrophysiologic assessment at the time of nerve explantation (24 weeks) to allow for a more precise assessment.

b. **Changes that had a significant impact on expenditures**
    i. Changing the site from the original Thorn animal facility to the animal facility at 221 Longwood Avenue (ICE lab) incurred significant costs due to staffing and operating room use. This was previously detailed in an earlier report and as an amendment to the protocol. There is no OR charge in the Thorn facility where the original protocol was written to occur, whereas in the 221 Longwood Avenue OR facility, there is an hourly charge ($185/hour; plus 60% overhead of $143.38) in addition to staffing charges associated with animal care and anesthesia. This was necessary due to additional space both in the OR and in the housing facility at 221 Longwood avenue for all of our animals (18) and time (24 weeks).
c. **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
d. **Significant changes in use or care of human subjects –**
    i. Not applicable
e. **Significant changes in use or care of vertebrate animals.**
    i. Nothing to report
f. **Significant changes in use of biohazards and/or select agents**
    i. Not applicable

6. PRODUCTS:

a. **Publications, conference papers, and presentations**
    i. **Journal publications.**
        1. Aycart MA, Alhefzi M, Bueno E, Pomahac B. Surgical Anatomy of the Whole Facial Nerve for Enabling Craniofacial and Regenerative Medicine Translational Research in Swine. Journal of reconstructive microsurgery 2015;31:547-50.
            a. Status: Published
            b. Acknowledgement of federal support: Yes
    ii. **Books or other non-periodical, one-time publications.**
        1. Nothing to report
    iii. **Other publications, conference papers, and presentations.**
        1. Aycart MA, Alhefzi M, Bueno EM, Pomahac B. Functional and Histological Evaluation of a Novel Branched Acellular Nerve Allograft and Processed Human Xenograft with and without FK506 in a Complex Branching Facial Nerve Defect: A Preliminary Study in Swine. Presented at the 2017 American Society for Peripheral Nerve Annual Meeting program, January 13-15, 2017. Waikoloa, Hawaii.

a. Acknowledgement of federal support: Yes

b. **Website(s) or other Internet site(s)**
   i. Nothing to report.
c. **Technologies or techniques**
   i. Nothing to report.
d. **Inventions, patent applications, and/or licenses**
   i. Nothing to report.
e. **Other Products**
   i. Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	*Bohdan Pomahac, MD*
Project Role:	*PI*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	12
Contribution to Project:	*Expertise in CTT, nerve surgery providing guidance in surgical and technical aspects of the project*
Funding Support:	*n/a*

Name:	*Mario Aycart, MD*
Project Role:	*Postdoctoral fellow*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*30*
Contribution to Projec	*Carried out all experiments, surgeries, nerve conduction studies and post operative care of the animals. Analysis of the data, annual and final technical report writing*
Funding Support:	*n/a*

Name:	*Muayyad Alhefzi, MD*
Project Role:	*Research assistant*
Researcher Identifier (e.g. ORCI ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Assisted in surgeries and in the post-operative care of animals*
Funding Support:	*n/a*

Name:	*Curt Deister, PhD*
Project Role:	*Investigator at AxoGen site*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked	*12*
Contribution to Project:	*Senior AxoGen scientist. Processing allografts and xenografts. Product optimization.*
Funding Support:	*n/a*

a. **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
   i. Nothing to Report
b. **What other organizations were involved as partners?**
   1. **Organization Name:** AxoGen, Inc. (AXGN)
   2. **Location of Organization:**
   3. 13859 Progress Blvd. Suite 100, Alachua, FL 32615
   4. **Partner's contribution to the projec**t
      a. Collaboration - whereby AxoGen was tasked with acquisition of both human and porcine cadaveric, branched peripheral nerves, decellularization of branched peripheral nerves and final processing, sterilization and Quality Assurance testing

8. SPECIAL REPORTING REQUIREMENTS

a. **COLLABORATIVE AWARDS:**
   i. Not applicable

9. APPENDICES: